transient increase of the intracellular Ca^{2+} ions. Our ultimate goal is to elucidate mechanotransduction mechanisms of chondrocytes both in healthy cartilage and in cartilage damaged by OA. Our objective of this study is to characterize the mechanical loading induced Ca^{2+} influx of chondrocytes, and to verify the key components contributing to chondrocytes' mechanosensitivity by using selected chemical compounds (Thapsigargin, GSK205, Ruthenium red, FM1-43, Cytochalasin-D, dynasore). Methodologically, we employ ratiometric calcium imaging, Atomic Force Microscope (AFM) and electrophysiology techniques. Our result reveals that chondrocytes are mechanosensitive cells. Typically, we observe individual chondrocytes taking up Ca^{2+} at ~600 nM, where basal level of ~100 nM Ca^{2+}, in response to mechanical loading of ~300 nN. The Ca^{2+} influx is mostly from extracellular rather than internal sources. In this meeting, we will introduce our combined measurement platform of AFM and Ca^{2+} imaging, and present detailed data of the molecular mechanisms governing mechanically-induced transient Ca^{2+} influx of chondrocytes.

1968-Pos Board B698
Using Atomic Force Microscopy to Probe Microalgal Response
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Microalgae are extremely abundant and important microorganisms, which affect a variety of environmental factors. Microalgae create almost half of the oxygen in the atmosphere and also sequesters greenhouse gases, like carbon dioxide, in order to grow. Microalgae can be exposed to diverse environmental stimulations, which affect their response. Here, we investigate the environmental stimulation mode of mechanics which is directly related to their environment such as fluid flow. We mechanically stimulate single Scenedesmus dimorphus cells and understand how this affects their structural response. To accomplish this, we use atomic force microscopy (AFM) to image S. dimorphus while simultaneously capturing optical images of the cell response. This integrated approach allows us to map the AFM mechanical measurements to specific subcellular locations on the individual cells. We were then able to perform force measurements with the AFM to determine properties such as Young's modulus of S. dimorphus. These findings are enabling us to understand mechanical properties of a single Scenedesmus dimorphus cell, which will empower us to map these responses to environmental stimulation and optimize their environmental benefits.

1969-Pos Board B699
The Effect of Disease and Exercise on Single Fibrin Fiber Mechanical Properties
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Fibrin fibers are the major structural component of a blood clot. Their properties affect wound healing and diseases, such as thrombosis, heart attacks and strokes. Working with purified fibrinogen, we have found that fibrin fibers have extraordinary extensibility and elasticity1,2. Recently, we have studied the more complex and more physiologically relevant fibrin fibers in plasma clots, in an effort to find relationships between single fibrin fiber mechanical properties and diseases. We determined the mechanical properties of single fibrin fiber of individuals who have cardiovascular disease (CVD), diabetes, or who have undergone an acute bout of strenuous exercise.
We found that fibrin fibers from old individuals with CVD are much more stretchable (~1.5 times), elastic (~1.4 times) and much stiffer (higher modulus) than those from healthy people. Moreover, we found that acute exercise also has a significant effect on fibrin fiber mechanical properties; fibrin fiber extensibility decreases significantly after exercise. Diabetes does not have a significant effect on single fibrin fiber mechanical properties. However, in the diabetes data, and subsequently in all other samples, we saw a startling correlation between fiber diameter and fiber stiffness: Fibrin fiber modulus decreases as the diameter of the fiber increases. For most samples, the modulus varied as R-1.3 or R-1.4, except for older individuals with cardiovascular disease, where the modulus varied as R-1.0. We propose a model in which the density of fibrin fibers varies: fibrin fibers have a dense core, and a less dense periphery.
1. Liu, W., et al. (2010) ''The mechanical properties of single fibrin fibers'', J. Thrombosis and Haemostasis 8, 1030-1036.
2. Liu, W., et al. (2006) ''Fibrin Fibers have Extraordinary Extensibility and Elasticity'' Science 313, 634.

1970-Pos Board B700
Investigating Mechanical Properties of Short Polymers with Optical Tweezers
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Studying mechanical response of biological molecules at a microscopic level is crucial for better understanding their structure-function relationship and how it relates to their physiological performance. Our group uses optical tweezers to study mechanical properties of structural proteins such as collagen. Collagen is an extracellular matrix protein that serves as the main foundation of connective tissues with a major role in the structural support of cells. Being the most abundant protein in the body, collagen is important for development, tissue regeneration and repair. After decades of study, the flexibility of collagen remains unresolved, with estimates of persistence length ranging over an order of magnitude (15-160nm), and how and at what forces it denatures under applied tension revealed only indirectly. Direct measurements of its force-extension relationship, and their dependence on composition and chemical environment, will help to resolve these structural questions.
The contour length of collagen (~300nm) is short with respect to the micron-sized spheres used in our optical tweezers experiments. Because of this, factors negligible in the studies of longer polymers, such as lambda-DNA, become important here, including geometric offsets associated with binding and stretching directions. In order to characterize and eliminate these offsets in our stretching experiments, we adapt a method from surface-based assays [Seol et al. Biophys J, 93: 4360 (2007)] to our pipette-based manipulation experiments. By stretching at different offset positions perpendicular to the stretching axis, we find that the geometric pulling offset can be minimized. Applying this method to force-extension curves of short DNA, we find that this approach improves the confidence in worm-like chain fitting parameters. We then apply this method to characterizing the flexibility of type III collagen molecules, whose contour length is comparable to the 1kbp DNA used in our control experiments.

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The Effects of Histone Variant H2A.Z on Chromatin Structure and Kinetics-A Single Molecule Optical Tweezers Study
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Understanding how histone variants affect chromatin structure is essential for understanding their role in gene regulation and chromatin maintenance *in vivo*. The basic unit of chromatin is a nucleosome, which consists of 146 base pairs of DNA wrapped around a histone octamer, made up of two of each of the four core histones-H2A, H2B, H3, H4. Often in cells, however, these canonical histones get exchanged for histone variants so that certain regions of the genome become enriched with non-wild-type histones. For example, histone variant H2A.Z has been found to replace canonical H2A near the transcription start site of active genes, suggesting that H2A.Z promotes gene transcription. Conversely, H2A.Z has also been associated with regions of silent chromatin. In general, the mechanism by which H2A.Z affects chromatin structure and kinetics is not understood. Here, we use optical tweezers to mechanically stretch individual nucleosome arrays and quantitatively compare the behavior of nucleosomes containing wild-type histones and nucleosomes in which histone H2A has been replaced with H2A.Z. We find that H2A.Z decreases the nucleosome inner-turn rewinding rate compared to wild-type H2A, promoting an open conformation. Our results reveal the affect of H2A.Z on nucleosome kinetics and on chromatin structure.

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Experimental and Simulation Studies on the Mechanical Properties of Sumo Proteins
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Protein structure plays an important role in determining its mechanical stability. In order to understand the role of amino acid sequence on the mechanical stability, mechanical unfolding experiments were performed using atomic force